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THE EFFECTS OF CALCIUM ON SEROTONIN-STIMULATED ADENYLATE CYCLASE IN FRESHWATER MUSSELS

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ABSTRACT

When serotonin (5-HT) is added to a pondwater bath (final concentration, 10^{-4} *M*/l) containing freshwater mussels, sodium influx is rapidly stimulated, as are behavioral responses. Serotonin also stimulates adenylate cyclase (AC) in gill homogenate pellets from two different freshwater bivalve families, suggesting that a serotonin-cAMP system is associated with control of Na transport and that this system may be common in many ion regulating mussel species. Adenylate cyclase activity was detected in several tissues of *Ligumia subrostrata;* the highest activity being observed in the foot. Endogenous calcium depressed the AC activity measured under basal, 5-HT, and dopamine stimulated conditions; the reduced enzyme activity was most pronounced in the monoamine stimulated preparations. AC activity of the crude homogenate pellet was significantly lower than a purified pellet formed by an additional $100 \times g$ centrifugation prior to a $3000 \times g$ centrifugation. Exogenous calcium (2.5 m*M*/l) inhibited monoamine stimulated AC activity about 50%. Calcium concretions exist in gill tissue and may influence the observed AC activity by increasing the protein measure or increasing the calcium concentration. Prostaglandin E_2 had no effect on basal or 5-HT stimulated AC activities in the purified pellet. Although phosphodiesterase and non-specific phosphatase activities were high in the supernatant, their activities in the homogenate pellet were low and had little effect on the AC activity measurements.

INTRODUCTION

Freshwater mussels maintain blood ion concentrations (Na, Cl, Ca, and HCO_3) above the level in pondwater (Dietz, 1979). Sodium and chloride transport are independent of each other in freshwater mussels (Scheide and Dietz, 1982); the gill is the primary site of Na accumulation (Dietz and Findley, 1980; Dietz and Graves, 1982). Although the mechanisms regulating each of these ions have not been elucidated, sodium ion regulation appears to be one function of a serotonin (5-HT)-coupled adenylate cyclase system in freshwater bivalves (Dietz *et al.,* 1982; Scheide and Dietz, 1983). Thus, a 5-HT stimulated adenylate cyclase (AC) is present in mussel gill tissue; a 5-HT dose-related effect on sodium influx has been observed; and gill cAMP levels are directly related to sodium transport rates (Dietz *et al.,* 1982; Scheide and Dietz, 1983).

The AC activity of *Ligumia subrostrata* gill tissue varies from mussel to mussel (Scheide and Dietz, 1983). Adenylate cyclase is a calcium sensitive enzyme, but the sensitivity is biphasic with 1–100 μM/l calcium activating AC whereas 0.1 to 1.0 m*M*/l inhibits the enzyme (Hynie and Sharp, 1971; Bockaert *et al.,* 1972; Brostrom

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et al., 1977). Since calcium concretions occur in the gills (Silverman *et al.*, 1983), we examined the effects of Ca on AC activity in mussel gill homogenate as a possible source of AC variability.

In this report, we present information on sodium transport stimulation by serotonin in several species of freshwater mussels and relate this to the 5-HT stimulated adenylate cyclase. We also present data on the 5-HT stimulated AC in several tissues in *Ligumia subrostrata*.

Materials and Methods

All organic chemicals were from Sigma Chemical Co. The ^{22}Na used in flux determinations was from New England Nuclear and the ^{3}H-cAMP (26 Ci/m*M*) for the binding protein assay was from Amersham.

Representative members of the Unionidae (*Anodonta grandis, Carunculina texasensis, Ligumia subrostrata*) and Corbiculidae (*Corbicula fluminea*) were collected locally and stored in aerated artificial pond water (0.5 m*M*/l NaCl, 0.4 m*M*/l $CaCl_2$, 0.2 m*M*/l $NaHCO_3$, and 0.05 m*M*/l KCl). These bivalves were acclimated to laboratory conditions for at least five days prior to experimentation. Males were used when possible to avoid interference by the brooding of glochidia.

Blood samples were taken by cardiac puncture and from the ventral edge of the foot. Handling mussels when the foot is extended results in a stream of blood from a "hemal pore" in the foot (see Greenaway, 1970). This blood was collected by directing the fluid into a centrifuge tube. Blood collected by cardiac puncture or from the foot was centrifuged for 1 min at 8000 × *g* and analyzed by flame photometry (sodium), electrometric titration (chloride), and atomic absorption spectrophotometry (calcium).

Sodium fluxes were analyzed to show the immediate effect of serotonin (5-HT) on sodium transport. Bivalves were rinsed for about 1 h in distilled H_2O (DW) and then placed in individual containers of pondwater containing ^{22}Na (Graves and Dietz, 1982). Once the animals had opened and had commenced siphoning, flux determinations were started with the removal of a bath sample. Basal flux periods averaged about two hours; then serotonin (final bath concentration of 10^{-4} *M*/l) was added to the pondwater medium and mixed gently to minimize the disturbance to the animals. Within 15 min the bivalves had opened again and were siphoning and the serotonin flux measurement was initiated by withdrawing a bath sample. The average flux period during 5-HT stimulation was one hour, then the final pondwater bath sample was removed.

Sodium net flux was determined by monitoring the change in the sodium concentration of the bath for several hours. The bivalve soft tissue was removed, dried at 90°C, and weighed. The net flux was expressed as μeq (g dry tissue · h)$^{-1}$. Sodium influx was determined by monitoring the disappearance of ^{22}Na from the pondwater bath; the efflux was calculated by: $J_{out} = J_{in} - J_{net}$.

The adenylate cyclase assay is detailed in Scheide and Dietz (1983). Briefly, tissue was homogenized in 5 ml 50 m*M*/l tris-SO_4, pH = 7.6 buffer with a Tissumizer (Tekmar) and centrifuged at 3000 × *g* for 20 min at 4°C. The surface of the crude homogenate pellet was rinsed with 2.0 ml tris-SO_4 buffer and then resuspended in 5 ml buffer. A purified pellet was produced as follows. The crude homogenate was first centrifuged at 100 × *g* for 20 min (4°C). The supernatant was decanted and recentrifuged 20 min at 3000 × *g*. The resulting purified pellet was rinsed in 2 ml of buffer and resuspended in 5 ml buffer. This procedure removed the large cells, cellular fragments, and the calcium concretions (see Silverman *et al.*, 1983).

Adenylate cyclase activity was determined by incubating the resuspended pellet, at room temperature, in a mixture of 50 m*M* tris-SO_4, pH = 7.6, 10 m*M* $MgSO_4$, 2 m*M* theophylline, 0.5 m*M* ethylenediamine tetraacetic acid (EDTA), 0.5 m*M* adenosine 5′ triphosphate, 0.1 m*M* guanosine 5′ triphosphate, 2.5 m*M* creatine phosphate, 2.5 units per test tube creatine phosphokinase (25°C), and other additions as noted in the Results (neurotransmitters and $CaCl_2$). The reaction was initiated by adding the resuspended pellet to give a total incubation volume of 200 μl; it was terminated after 5 min by adding 0.8 ml boiling water and boiling the reaction tube for 3–5 min. Cyclic AMP formed during the assay was quantified with a cAMP binding protein isolated from human red cells (Scheide and Dietz, 1983). Adenylate cyclase activity was expressed as pmol cAMP (mg protein · 5 min)$^{-1}$. Protein values were determined by the Lowry method (Lowry *et al.,* 1951).

The AC activity was determined in homogenates of gills from the 4 species studied. Homogenates were resuspended to give between 0.12 and 0.17 g original wet gill tissue weight per ml of resuspended pellet. Dilution of various *Ligumia subrostrata* tissue crude homogenate pellets averaged 0.13 to 0.21 g original wet tissue weight per ml of resuspended pellet for gill, mantle, foot, heart, and muscle.

Endogenous calcium values were determined by centrifuging the resuspended homogenate pellet at 8000 × *g* for 1 min and diluting a known volume of supernatant in 1% LaO_3–5% HCl. This procedure separated the particulate calcium concretions in the homogenate from the "soluble" supernatant calcium. Calcium was measured with a Perkin-Elmer 303 atomic absorption spectrophotometer.

Cyclic AMP degrading enzymes were assayed by incubating 300 pmol cAMP in a reaction tube with a final concentration of 50 m*M*/l tris-NO_3, pH = 7.6, 10 m*M*/l $Ca(NO_3)_2$. Gill tissue from *Ligumia subrostrata* was homogenized in 3–5 ml 50 tris-NO_3, pH = 7.6 buffer. Tissue fractions were obtained after a 3000 × *g* centrifugation for 20 min (4°C). The pellet was resuspended in tris-NO_3 buffer, 1 ml per 0.21 g wet tissue weight. The supernatant was from a homogenate formed by 0.14 g wet tissue weight per ml of homogenate buffer. The phosphatase assay was initiated by the introduction of the supernatant fraction (final volume, 0.2 ml) and terminated by the addition of 0.8 ml boiling water followed by boiling the reaction tube for 3–5 min. The cAMP remaining was determined by use of the cAMP binding protein (Scheide and Dietz, 1983). A zero activity control was prepared by boiling (3–5 min) the supernatant fraction (diluted with 0.8 ml DW), then adding tris-NO_3/$Ca(NO_3)_2$ buffer and cAMP. Protein determinations were by the method of Lowry *et al.* (1951). Enzymatic activities were determined by subtracting the cAMP concentration measured in each reaction tube from that of the zero activity tube and were expressed as pmol cAMP degraded (mg protein · 5 min)$^{-1}$. All assays were done in triplicate. Phosphodiesterase activity was determined by subtracting the cAMP present per reaction tube containing 4.0 m*M* theophylline from the cAMP remaining in a non-theophylline containing reaction tube. The tube with theophylline represented non-specific phosphatase (referred to as phosphatase) activity. Preliminary results indicated this reaction to be linear for the first 10 min.

Data are expressed as mean ± standard error. Statistical analyses utilized were the two-tailed Student's *t*-test and least-squares linear regression.

Results

Sodium transport in the four species was stimulated by 5-HT (10^{-4} *M*/l in pondwater) (Table I). All four species exhibited the same "serotonin response" observed previously when 5-HT was injected into the foot of the mussel (Dietz *et al.,* 1982).

TABLE I

Control and serotonin (10^{-4}M/l) stimulated sodium transport in four representative mussels immersed in pondwater

		SODIUM FLUX μeq (g dry tissue · h)$^{-1}$					
		Control			5-HT Stimulated		
Species	n	J_{net}	J_{in}	J_{out}	J_{net}	J_{in}	J_{out}
Anodonta grandis	6	-0.27 ± 0.35^{A}	0.45 ± 0.29	0.72 ± 0.63	-1.63 ± 0.63	2.29 ± 0.66^{B}	3.92 ± 0.33^{C}
Carunculina texasensis	6	-0.41 ± 0.10	0.63 ± 0.20	1.04 ± 0.19	1.55 ± 0.32^{C}	2.01 ± 0.32^{CE}	0.46 ± 0.16^{B}
Corbicula fluminea	5	-1.89 ± 0.93	4.62 ± 0.85	6.51 ± 1.52	8.18 ± 0.94^{D}	10.59 ± 1.28^{CE}	2.41 ± 0.65^{B}
Ligumia subrostrata	5	0.67 ± 0.83	1.18 ± 0.65	0.51 ± 0.25	5.00 ± 0.50^{D}	5.60 ± 0.77^{CE}	0.60 ± 0.32

[A] Values represent the mean ± the standard error of the mean.
Values significantly different from controls, B: $P < 0.05$
C: $P < 0.01$
D: $P < 0.001$.
Values of J_{in} and J_{out} significantly different E: $P < 0.01$.

The control flux of each individual was determined immediately before the addition of 5-HT.

The reaction to serotonin treatment includes an increase in siphoning, pronounced valve gaping movements, an extension of the foot, and increased locomotor activity. The 5-HT stimulated sodium influx in each species was 2.5 to 5 fold over the basal sodium influx. In *Carunculina texasensis, Corbicula fluminea,* and *Ligumia subrostrata* 5-HT stimulated the influx significantly above the efflux resulting in a significant ($P < 0.01$) net movement of sodium into the mussel. However, in *Anodonta grandis,* the sodium efflux was significantly increased ($P < 0.01$), in addition to the stimulation of the influx, resulting in a net loss of sodium. The loss of sodium by *A. grandis* could have been due to a serotonin effect on the excretory component, a loss of fluid from the foot during valve gaping and locomotor movements which occurred throughout the flux interval, or from the disturbance associated with bath sample withdrawal.

Analysis of the foot blood (see Materials and Methods) indicated that although its ion concentrations were approximately 47% and 64% of those in the blood of *A. grandis* and *L. subrostrata,* the ratios of the ions in the two fluids were similar (Table II). The low concentrations in the expelled fluid and the high sample variability probably resulted from dilution by pondwater present in the mantle cavity and gill chamber. On two occasions when a fluid stream was directed horizontally and collected in a beaker held several cm from the animal, the Na concentration was higher than the fluid dripping directly from the ventral edge of the foot (18.8 ± 0.1 meq Na/l for *A. grandis,* n = 2). The Na, Cl, and Ca concentrations in the fluid collected from the foot were between 5 to 17 times greater than those in the pondwater.

Adenylate cyclase activity was measured in the resuspended crude homogenate pellet of gill tissue from the four mussel species (Fig. 1). Serotonin increased the AC activity ($P < 0.01$), by 3–5 times the basal level in *A. grandis, C. fluminea,* and *L. subrostrata.* In *C. texasensis* the increase in AC activity by 5-HT was greater than 30 times the basal level; but the enzyme activities produced by 5-HT stimulation were similar in all of the species. Basal AC activity levels were significantly lower ($P < 0.01$) in *C. texasensis* when compared to basal values of *A. grandis, C. fluminea,* and *L. subrostrata.* The trends in AC activity, normalized to Lowry protein concentration, were essentially unchanged when AC activity was expressed per gram wet tissue. *Anodonta grandis, C. fluminea,* and *L. subrostrata* had similar gill homogenate pellet AC basal and 5-HT stimulated activities: *A. grandis,* 1158 ± 289 *versus* 3658 ± 925 (n = 6); *C. fluminea,* 1147 ± 319 *versus* 4384 ± 1048 (n = 4); and *L. subrostrata,* 1499 ± 202 *versus* 3566 ± 439 (n = 6) pmol cAMP (g wet tissue · 5 min)$^{-1}$. *Carunculina*

TABLE II

Blood and foot ventral edge fluid collected from Anodonta grandis *and* Ligumia subrostrata *after a 1.5 h exposure to* 10^{-4} M/l *5-HT pondwater*

	ION CONCENTRATION mM/l							
	Blood				Foot Fluid			
Species	n	Na	Cl	Ca	n	Na	Cl	Ca
Anodonta grandis	10	19.3 ± 0.7[A]	17.0 ± 0.6	3.7 ± 0.2	4	12.2 ± 4.0	10.2 ± 3.6	2.6 ± 0.7
Ligumia subrostrata	11	20.1 ± 0.3	13.7 ± 0.4	3.5 ± 0.1	4	9.2 ± 1.9	6.2 ± 1.6	1.7 ± 0.3

[A] Values represent the mean ± the standard error of the mean.

Pond water values for the ions in this table were 0.7, 1.2, and 0.3 mM/l for Na, Cl, and Ca, respectively.

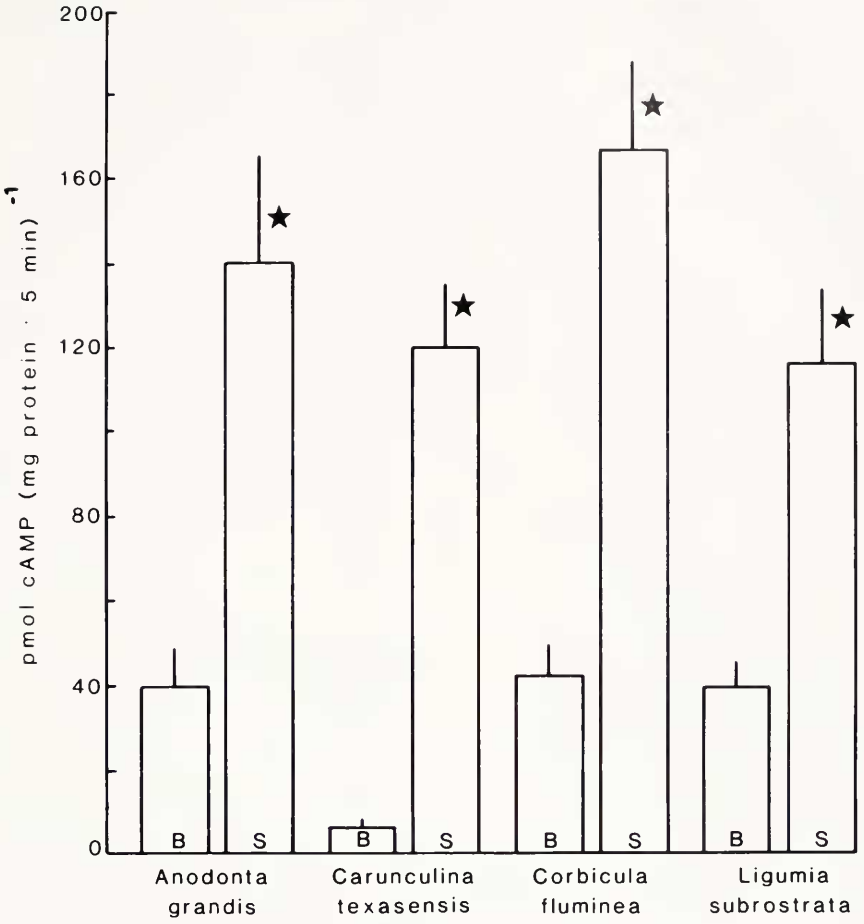

FIGURE 1. Basal (B) and 5-HT (S) (60 μM/l) stimulated AC activity in four species of freshwater mussels. Each corresponding bar represents the mean of five *Anodonta grandis*, four *Carunculina texasensis*, four *Corbicula fluminea*, and six *Ligumia subrostrata* gill tissue crude homogenate pellets assayed in triplicate. The vertical lines represent the standard errors of the mean. ★ = significantly different from corresponding basal values, $P < 0.01$.

texasensis exhibited 75.9 ± 46.5 *versus* 2828 ± 391 pmol cAMP (g wet tissue · 5 min)$^{-1}$ for basal and 5-HT AC activities (n = 4).

Adenylate cyclase was present in all *L. subrostrata* tissues studied (Fig. 2). Serotonin is a major neurotransmitter substance in bivalves and it significantly stimulated AC activity in each tissue. Basal AC activities observed in the gill, mantle, heart, and posterior adductor muscle were similar as were the 5-HT stimulated activities, displaying a 2.2 to 4.6 fold increase over basal AC activity. However, the foot muscle basal and the 5-HT stimulated AC activities were significantly higher ($P < 0.02$) than those in the other tissues. The same trends were evident whether AC activities were expressed in terms of gram wet tissue weight or normalized to Lowry protein content.

Adenylate cyclase activity values are quite variable between animals (this study, Scheide and Deitz, 1983). To better understand this variability, the relationship between AC activity and the endogenous calcium content was studied (Fig. 3). Observed AC

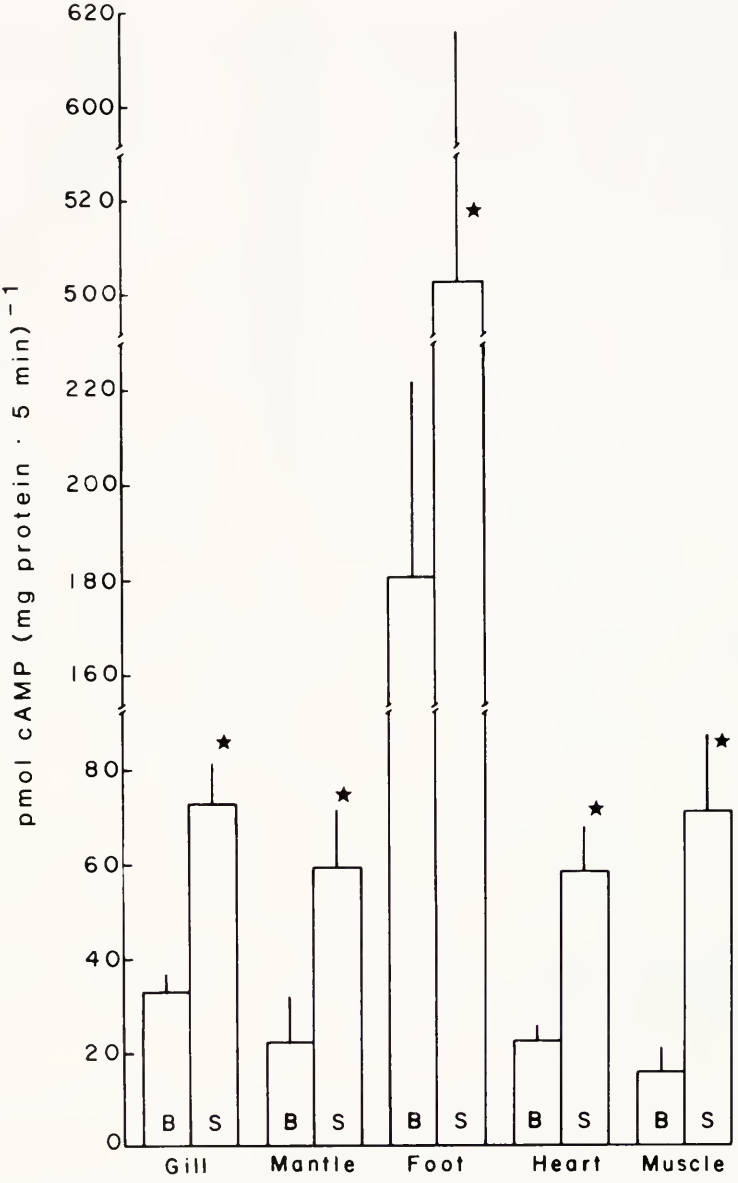

FIGURE 2. Basal and 5-HT (60 μM/l) stimulated AC activity in the various tissues of *Ligumia subrostrata*. The bars represent the mean of seven gills and three each of mantle, foot, heart, and posterior adductor muscle, each assayed in duplicate using crude homogenate. Vertical lines represent the mean standard error. ★ = significantly different from corresponding basal values, $P < 0.05$.

activity was inversely dependent on the endogenous calcium in the homogenate pellet. Basal ($r = 0.77$, $P < 0.001$), serotonin ($r = 0.88$, $P < 0.001$) and dopamine ($r = 0.79$, $P < 0.001$) stimulated AC activities were inversely related to endogenous calcium with the calcium appearing to have a greater effect on the monoamine (5-HT and dopamine) stimulated activity than the basal activity. Basal AC activity *versus* en-

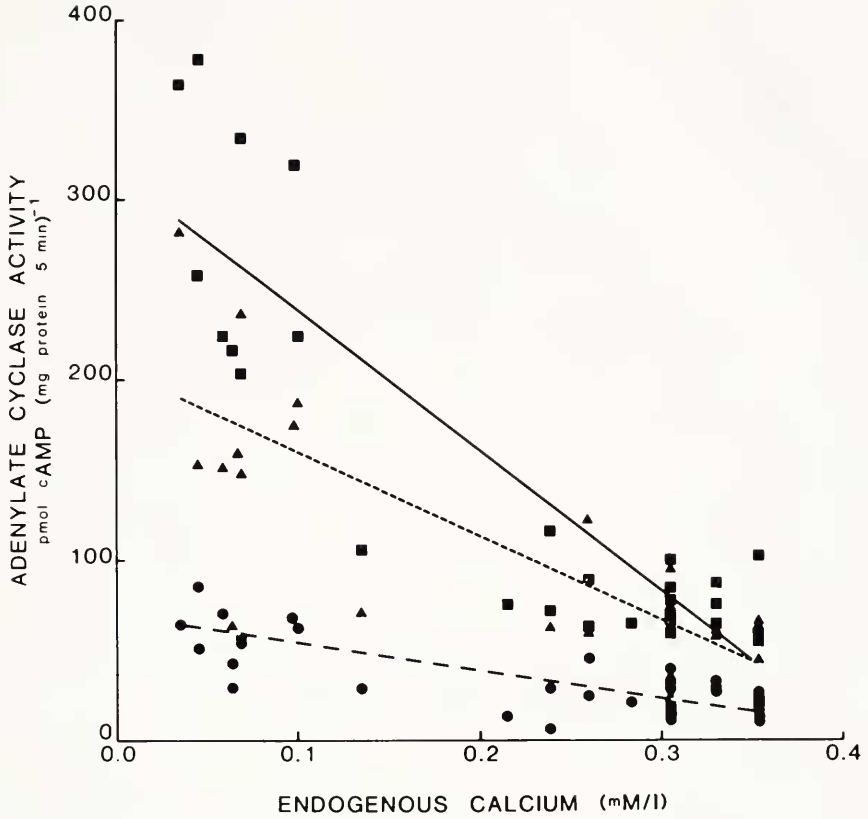

FIGURE 3. Gill adenylate cyclase activity varies inversely as a function of endogenous calcium in *Ligumia subrostrata*. Each point represents the mean value of three determinations. ● = basal (n = 33), ▲ = 10 μM/l dopamine (n = 24), and ■ = 10 μM/l serotonin (n = 32) AC activities with the lines fit by linear regressions.

dogenous Ca linear regression was calculated to have a slope of −130 pmol cAMP (mg protein · 5 min)$^{-1}$/(mM Ca/l) while slopes of both 5-HT and dopamine stimulated activities were higher (−763 ± 75 and −463 ± 19 [pmol cAMP (mg protein · 5 min)$^{-1}$]/(mM Ca/l), respectively). The effect of calcium on the monoamine stimulated activities was significantly different ($P < 0.01$) from basal; the most pronounced effect was produced by 5-HT.

Addition of exogenous calcium ($CaCl_2$) inhibited purified pellet AC (Fig. 4). The effect of exogenous calcium was primarily on the monoamine stimulated AC activities. Monoamine stimulated AC activities were significantly inhibited ($P < 0.01$). Normally, 5-HT and dopamine stimulated AC activities were 4.8 and 3.2 times above basal, respectively; in the presence of $CaCl_2$, monoamine stimulated AC activity (above $CaCl_2$ basal) was only 3.5 and 2.0, respectively. Basal AC activity also was reduced by the addition of $CaCl_2$. The addition of exogenous calcium (50% basal AC inhibition at a concentration 2.5 mM/l) was not as effective as the endogenous calcium (about 0.25 mM/l calcium).

The phosphodiesterase and non-specific phosphatase activities of the crude homogenate pellet and the supernatant were studied to determine whether these enzyme

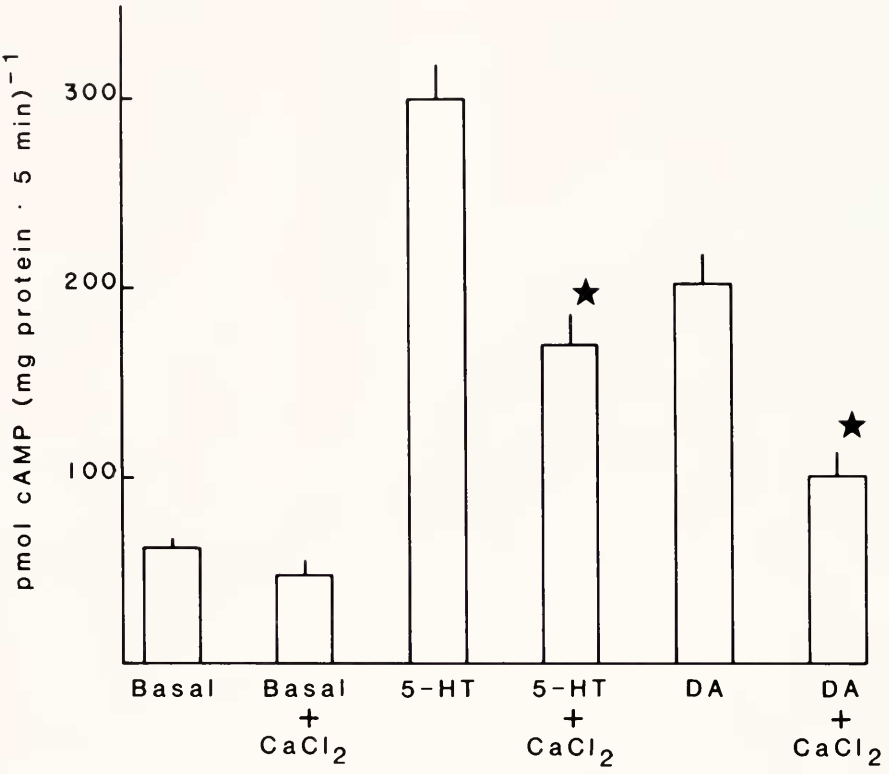

FIGURE 4. *Ligumia subrostrata* gill AC activity of basal, 10 μM/l 5-HT, and 10 μM/l dopamine with or without 2.5 mM/l $CaCl_2$. Each bar represents the mean of four gills, each assayed in triplicate using purified pellet. The vertical lines indicate the mean standard error. ★ = significantly inhibited from preparations without added Ca, $P < 0.01$.

activities could be correlated with the variability exhibited by adenylate cyclase in *Ligumia subrostrata* gills (Fig. 5). Phosphodiesterase and phosphatase activities were similar within pellet but differed in the supernatant fractions, however the activities of the two fractions were significantly different ($P < 0.001$) from each other. Supernatant phosphatase activity was 4.5 times higher than that observed in the pellet, whereas phosphodiesterase was 2.5-fold higher. The summation of both enzyme activities was 267 ± 67 pmol cAMP degraded (mg protein · 5 min)$^{-1}$ (n = 9) in the pellet fraction and a significantly higher ($P < 0.001$) 1038 ± 213 pmol cAMP degraded (mg protein · 5 min)$^{-1}$ in the supernatant fraction. Since the enzymes catabolizing cAMP were measured under optimal conditions, their activity in the pellet fraction appears to be too small to have an appreciable effect on the cAMP concentrations present in the AC activity assay. Rarely would the AC produced cAMP exceed 2 pmol in our assay conditions and sufficient theophylline was present to inhibit phosphodiesterase activity.

Prostaglandin E_2 inhibits sodium influx (Graves and Dietz, 1982; Saintsing and Dietz, 1983) and could possibly inhibit adenylate cyclase. The addition of PGE_2 did not effect basal purified pellet AC activity. In the presence of 50 ng/ml (140 μM/ml)

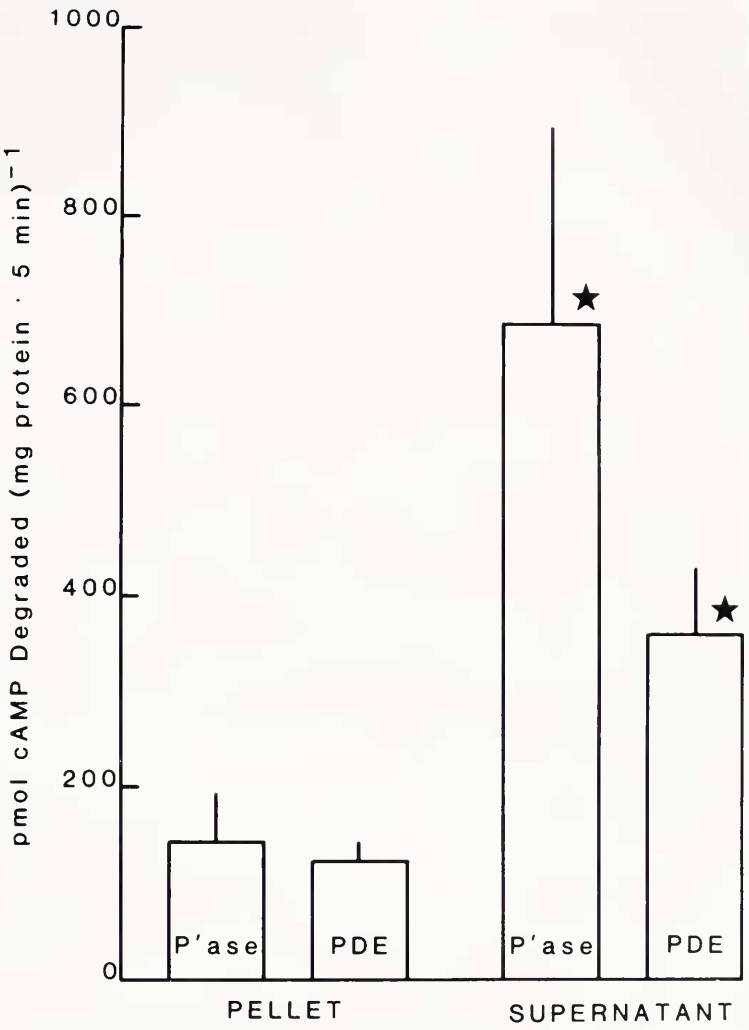

FIGURE 5. Nonspecific phosphatase and phosphodiesterase activities in the crude homogenate pellet and the supernatant fractions formed by a 3000 × *g* centrifugation of *Ligumia subrostrata* gill homogenate. Bar values represent the mean of nine gill tissues for the pellet fraction and three gill tissues for the supernatant fraction, each assayed in triplicate. Vertical lines represent the mean standard error. ★ = significantly different from the same enzyme system in the pellet, $P < 0.001$.

PGE_2, AC activity was 68.4 ± 2.7 while basal was 57.5 ± 4.0 pmol cAMP (mg protein · 5 min)$^{-1}$ (n = 2 gills each assayed in triplicate). Additionally, the inclusion of PGE_2 did not effect 5-HT or dopamine stimulated AC activity.

DISCUSSION

In freshwater mussels the sodium influx is stimulated by 5-HT and is correlated with the presence of a 5-HT stimulated adenylate cyclase. Basal flux values presented

are consistent with sodium transport values previously reported (Dietz, 1979), while the 5-HT stimulated values compare with those observed in salt-depleted mussels (Murphy and Dietz, 1976; Dietz, 1978; McCorkle and Dietz, 1980; Scheide and Dietz, 1982). We have reported that the sodium influx in non-salt-depleted *L. subrostrata* and *C. texasensis* increases, when the mussels are injected with dibutyryl cAMP, 5-HT, and dopamine (Dietz *et al.*, 1982; Graves and Dietz, 1982). In addition, *L. subrostrata* gill tissue has both 5-HT- and dopamine-stimulated AC activity (Scheide and Dietz, 1983). Although both 5-HT and dopamine increase cAMP in the gill epithelium (Scheide and Dietz, unpub.), and both control gill ciliary motility (Paparo and Murphy, 1975, Paparo, pers. comm.), only serotonin stimulates sodium uptake by a cAMP-dependent transport system in isolated gills (Dietz and Graves, 1981; Dietz *et al.*, 1982). Since 5-HT stimulates Na transport and also stimulates gill AC activity in two different bivalve families (Unionidae and Corbiculidae), the serotonin-adenylate cyclase coupled sodium transport may be present in most ion regulating bivalves.

Serotonin stimulated AC activity was present in all tissues investigated in *Ligumia subrostrata*. Serotonin is a ubiquitous neurotransmitter substance in bivalves (Hiripi, 1968, 1972; Sweeney, 1968; Smith, 1982), and 5-HT is responsible for many functions including: regulation of gill sodium transport (Dietz *et al.*, 1982), control of ciliary motility (Gosselin, 1961; Paparo and Murphy, 1975), muscle relaxation (Salanki and Hiripi, 1970; Painter, 1982), rhythmic activities (Hiripi and Salanki, 1973), and heart contraction (Higgins, 1974; Painter and Greenberg, 1982).

The comparatively high 5-HT stimulated AC activity in the foot muscle, when compared to the other tissues, may be due to adaptations for the control of burrowing and locomotion. Serotonin is a neuromuscular transmitter for a number of molluscan muscles (Painter, 1982; Vitellaro-Zuccarello *et al.*, 1983).

Calcium concretions in bivalve tissues may contribute to AC activity variability in several ways. Calcium bound to the concretions can become free in solution (Istin and Girard, 1970), thus inhibiting AC activities. Istin and Girard (1970) report two pools of calcium in mantle tissue; ionized and bound. Exchange between these pools occurs and may be inhibited by acetazolamide, implying carbonic anhydrase mediation. If a system similar to that found in the mantle occurs in the gill tissue, free calcium levels will increase in the resuspended homogenate pellet since carbonic anhydrase is present in gill tissue (Henry and Saintsing, 1983).

Calcium regulates AC activity (basal, 5-HT- and dopamine-stimulated) in the *in vitro* preparation and may contribute *in vivo*. Calcium (0.1–0.5 m*M*/l) inhibition of AC activity has been observed before (Hynie and Sharp, 1971; Marumo and Edelman, 1971; Bockaert *et al.*, 1972; Brostrom *et al.*, 1977; Litosch *et al.*, 1982; Schmidt *et al.*, 1982). Usually, AC inhibition occurs only when calcium is added to the system, but the endogenous calcium in mussel gills appears sufficient to inhibit the activity substantially. A source of endogenous calcium present in the resuspended crude homogenate pellet is the calcium concretions that contribute at least 25% of the dry gill weight (Silverman *et al.*, 1983). If all of the calcium concretions were solubilized in our crude homogenate pellet, the calcium concentration would be >60 m*M*/l. Separating the calcium concretions by centrifugation and analyzing the supernatant for Ca indicates the "soluble" calcium concentration is rarely greater than 0.4 m*M*/l. Gill AC levels are reduced by 70% at an endogenous "soluble" Ca concentration of 0.2–0.3 m*M*/l, but purified pellet AC activity is inhibited 50% by 2.5 m*M*/l exogenous Ca. In our studies, the specific mechanism of action of Ca cannot be determined. Calcium may modulate 5-HT and dopamine action at the AC receptor level or Ca

may decrease the basal AC activity due to the formation of Ca-ATP instead of Mg-ATP; the required substrate (Birnbaumer, 1973). However, we observed that the Ca effect was more pronounced on monoamine stimulated AC activity.

Calcium is typically associated with the activation of phosphodiesterase activity. Theophylline (in the concentration used in determining AC activity) inhibited 50% of the gill cAMP catabolic activity. Similar inhibition of phosphodiesterase activity was observed in *Mercenaria mercenaria* ventricle (Hess *et al.*, 1981). The remaining cAMP degrading activity was defined as phosphatase activity and should be constant in all similar preparations. In the gill homogenate pellet, the enzymatic degradation levels do not indicate that this cAMP catabolic activity significantly changes the AC activity measurements in bivalve tissue. The high level of cAMP catabolism occurring in the homogenate supernatant agrees with the previous observations that these degradative enzymes are found predominately in the soluble fraction: *e.g.*, mammalian skeletal muscle (Gain and Appleman, 1978) and cerebral cortex (Kakiuchi *et al.*, 1978). Supernatant AC activity, although greater than zero, was not significantly stimulated by 5-HT (Scheide and Dietz, 1983), perhaps masked by the substantial cAMP degradative enzymes.

Prostaglandin E_2 inhibits sodium transport and PGE_2 synthesis is inhibited by injections of serotonin or dibutyryl cAMP, thus reversing the PGE_2 effect (Saintsing *et al.*, 1983). However, crude homogenate pellet AC activity was unaffected by PGE_2 (Scheide and Dietz, 1983). Since the sodium influx stimulatory system (5-HT) and the inhibitory system (PGE_2) appear to be linked (Saintsing and Dietz, 1983), the purified pellet preparation was used to minimize the calcium interference. Addition of exogenous PGE_2 still did not significantly change AC activity from normal values; an observation similar to that reported for the rat outer medulla (Jackson *et al.*, 1980).

Sodium transport is positively modulated by a serotonin-cAMP system in freshwater bivalves. The Na regulatory system functions to maintain sodium balance during periods of ion depletion, water gain (the consequence of inhabiting a freshwater environment), or fluid loss. Fluid loss may be either through renal output or blood loss through "hemal pores" in the foot. We have noted a serotonin induced weight gain in mussels (unpub.) and the foot becomes engorged with blood. We have observed blood being expelled from the extended foot especially when the mussels are handled. Blood loss through "hemal pores" is documented in gastropods (Greenaway, 1970) and this blood loss may account for the observation that "mantle cavity fluid" is more similar to blood than pondwater (Matsushima and Kado, 1982).

Regulation of Na in freshwater mussels is a rapidly acting mechanism (initiated within 10 min) and may continue for several hours (Scheide and Dietz, 1983). The serotonin mechanism may be an integral part of the circadian rhythms exhibited by freshwater bivalves. Ion transport (Graves and Dietz, 1980; McCorkle-Shirley, 1982), as well as oxygen consumption and activity rhythms (Hiripi and Salanki, 1973; McCorkle *et al.*, 1979), exhibit coincidental phase relationships, being elevated during the dark phase and depressed during the light phase. Coordination of these activities is probably ganglionic, but at this time the site associated with monitoring sodium concentration and regulating blood Na is unknown.

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